

The University of Chicago

RELATION OF NUTRITION
OF TOMATO TO DISPOSITION TO
INFECTIVITY AND VIRULENCE
OF FUSARIUM LYCOPERSICI

A DISSERTATION SUBMITTED TO THE FACULTY
OF THE DIVISION OF THE BIOLOGICAL SCIENCES
IN CANDIDACY FOR THE DEGREE OF DOCTOR
OF PHILOSOPHY

DEPARTMENT OF BOTANY

1936

By
WILLIAM SCHLEI COOK

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RELATION OF NUTRITION OF TOMATO TO DISPOSITION TO INFECTIVITY AND VIRULENCE
OF *FUSARIUM LYCOPERSICI*

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 478

WILLIAM S. COOK

(WITH SEVEN FIGURES)

Introduction

The object of this investigation was to study the nutrition of the tomato as a factor in its disposition to infection by *Fusarium lycopersici*. The nutritional plane was varied through the supply of nitrates to the cultures kept under otherwise identical conditions. As the work progressed, a study of the browning of vascular bundles as the criterion of infection became essential.

Several investigators have studied the influence of fertilizers, or of some ingredients in the fertilizers, on infection by *F. lycopersici*, and the role of nutrition under controlled conditions of the substrate.

EDGERTON and MORELAND (5) found that the addition of large amounts of lime to the soil tended to hinder the rate of development of tomato wilt.

CLAYTON (3) found that plants grown in saturated soil were markedly different in their nitrogen relations from those grown in non-saturated soil, as indicated by microchemical analysis. Resistance seemed to be directly correlated with the absence of nitrate nitrogen in the soils. He tested this relationship by growing plants in sand cultures and adding nutrient solutions. Part of the plants received a complete nutrient solution and the remainder a solution lacking nitrates. Temperature conditions in the greenhouse did not permit a virulent development of the disease, but "it was conclusively shown that plants grown without nitrate, the tissues of which plants give no nitrate test, are not infected by the fungus. Plants grown with a complete nutrient solution were readily infected" (p. 142).

AHMET (1) studied the influence of a varied mineral nutrient sup-

ply upon the pathogenicity of *Fusarium lycopersici*. His experiments were carried out under greenhouse conditions in sand cultures. Because he obtained 100 per cent infection, clear differences were apparent only in incubation time. The potassium deficient plants showed the shortest incubation time and the earliest appearance of wilt (17 days), with death of the plants in 25 days. Phosphorus deficient plants were next, with 22 and 30 day periods respectively. Excess nitrogen plants followed with an incubation period of 26 days, and with death of the plants in 37 days. Next in order were plants given excess phosphorus and excess potassium. Last were the nitrogen deficient plants, which died at the end of 58 days, even though they did not wilt.

FISHER (6) grew plants of the Bonnie Best (susceptible) and Marglobe (resistant) varieties in sand culture under deficient and excessive amounts of the essential elements. Boron and nitrogen deficiencies inhibited fungous infection in the Bonnie Best variety. Its susceptibility was decreased when the plants were treated with excesses of calcium, magnesium, and phosphorus, and solutions lacking in magnesium and phosphorus. The resistance of the Marglobe variety was decreased when the plants were treated with solutions lacking calcium and potassium and excessive in potassium, nitrogen, and sulphur.

EDGERTON (4) studied the development of tomato wilt in the susceptible Acme and the resistant Louisiana Wilt Resistant varieties in pot cultures. Different soils were used, including river sand, garden soil (loess), and two mixtures, one of soil sand and leaf mold and another of soil and leaf mold. The various soils were sterilized, heavily inoculated with *F. lycopersici*, and seeds of the two varieties planted therein. Infection results were based both on the number of plants dead from wilt and on those plants showing signs of the disease. In river sand, Acme gave 20.0 per cent infection while Louisiana Wilt Resistant gave only 3.1 per cent infection. In soil made up of a mixture of sand and leaf mold, the susceptible variety gave 92.9 per cent infection while the resistant variety gave 72.2 per cent.

EDGERTON and MORELAND (5) recognized a progressive yellowing and necrosis of leaves up the stem, leading finally to the wilted condition. CLAYTON (2) recognized a progressive wilting of the leaves,

often accompanied or preceded by a yellowing of the affected leaves. In well developed plants three to four weeks old, the first external evidence of the disease was always wilting of a lower leaf. Under conditions which were not favorable for the maximum development of the disease (resistant plants, fungus lacking in virulence, or environmental conditions unfavorable to growth of either parasite or host), there was a tendency for the disease to appear as a slow blight rather than as a wilt. In this blight the leaves yellowed and died slowly. AHMET found that young infections led, according to the conditions and degree of infections, either to a chronic disease course or to an acute one. Infection of grown plants led mostly to the acute disease course. He also recognized that death of the infected plants may come about without true wilting. He recognized yellowing and curling of leaves, stunting of plants, and bleaching of stems as additional *Fusarium* symptoms. FISHER gives a new, not previously reported, external disease symptom to tomato wilt. He states: "Adventitious roots occur on the stem which keep step with the yellowing of the leaves as the fungus spreads up the stem."

Material and procedure

FUNGUS CULTURES.—Cultures of *Fusarium lycopersici* used in the experiments were obtained from Dr. G. K. K. LINK of the Department of Botany, University of Chicago, and from Dr. F. L. WELLMAN of the United States Department of Agriculture. The culture furnished by Dr. LINK was used for the first experiment, but as it was thought that this culture was lacking somewhat in virulence, the latter culture was obtained and used in all of the other experiments. In pot tests made in soil with these two cultures side by side, it was found that the second culture was somewhat more virulent, although the first gave good results.

NUTRIENT SOLUTIONS.—The composition of the nutrient solutions used is given in table 1, which gives the partial volume molecular concentrations of the salts.

CULTURE OF PLANTS.—The investigation was conducted in the greenhouses of the University of Chicago. Seeds of Bonnie Best (susceptible) and Marglobe (resistant) were germinated in rich black potting soil. When they were about 5 cm. high the seedlings

were transplanted into flats containing soil, about 100 to the flat. When the plants were 7.5-12.0 cm. in height, the most vigorous were removed and transplanted into soil in 10 cm. clay pots, one plant per pot, where they were allowed to develop until ready for use. The time from planting of seed until satisfactory plants were obtained varied, being about six weeks to two months in the spring and summer and at least three months in the winter. When cuttings were used, the plants were trimmed to two leaves per plant, cut at the base just above the soil line (see the various experiments for deviations), immediately placed in buckets of water, and transplanted into the sand culture pots, two plants per pot. After transplanting, they were protected by newspapers from the sun for several days.

TABLE 1
COMPOSITION OF NUTRIENT SOLUTIONS

SOLUTION	Ca (NO ₃) ₂	CaCl ₂	KH ₂ PO ₄	MgSO ₄
Plus nitrate.....	0.0090		0.0045	0.0045
Minus nitrate.....		0.0068	0.0045	0.0045

All of the experiments were carried out in sand cultures under controlled conditions, using soil-nutrient-temperature tanks (8). The aim was to grow the plants under environmental conditions most conducive to the type of growth desired. For the sand cultures, screened and sterilized white quartz sand was used; it was not subjected to any particular treatment of washing before use. An attempt was made to keep the sand below pH 6.0, and preferably at pH 5.5. Under such conditions the various salts are assimilated and the fungus grows readily. To attain this, all applications to the sand, either in the form of daily waterings with tap water or of nutrient solutions, were adjusted to pH 4.2.

Plants grown under plus nitrate culture received approximately 2.2 liters of plus nitrate solution per week, while those grown under minus nitrate culture received about 1.4 liters of minus nitrate solution during the same period. It was also necessary to flush with acidulated tap water once weekly in order to keep the concentration

of the salts in the sand below a possible toxic level. This aided in maintaining the desired acidity of the sand.

In all the experiments the temperature of the sand cultures was kept at 28° C. CLAYTON (2) found that this was the optimum temperature for growth of *Fusarium lycopersici*, and also very favorable for growth of the tomato. While he found that the air temperature was important in the development of the disease, the disease developing best under a soil temperature of 27° C. and an air temperature of 27° C., or equally as well under a soil temperature of 27° C. and an air temperature of 33° C., no attempt was made to control the air temperature in the greenhouses. The experiments extended through more than a year, with attendant variations in air temperature. Whenever possible air temperatures were kept at 21° C. during the night and at 25° C. during the day. Air temperatures up to 50° C. were recorded on some days.

INOCULATION OF PLANTS.—The ordinary plus nitrate nutrient solution in the several experiments served as an excellent growth medium for the *Fusarium* organism if supplemented with 3.3 per cent sucrose. In this solution an abundant growth was made in eight to ten days at ordinary room temperature. For cultural purposes, 250 cc. of the nutrient medium was placed in 500 cc. Erlenmeyer flasks, autoclaved, and later inoculated with a small amount of mycelium from a young culture of *F. lycopersici*.

The inoculum was either applied directly to the roots of the plants growing in sand (plants thus inoculated will be referred to hereafter as post-inoculated), or the sterile sand was inoculated previous to planting (plants referred to hereafter as pre-inoculated). In the former method the sand was pulled back from around the roots to the depth of 10–12 cm. and the contents of one culture flask (250 cc.) poured over the roots. In the latter method about 20 cm. of the sterilized sand was removed from the soil containers, the contents of a single culture poured in, and the removed sand replaced and mixed thoroughly with the inoculum. At various times during the experiments, isolations were made from the sand cultures to check the presence of the fungus. Results of these isolations showed that the organism grew well in the sand without the addition of carbohydrates.

SEEDLING TESTS.—Germination of seed and growth of seedlings took place under plus and minus nitrate nutrition. After germination, the seedlings were thinned to a definite number per pot. Infection results are based on the recovery of the fungus from any part of the stems of the seedlings. All isolations which showed fungous growth were checked microscopically.

CRITERIA OF INFECTION.—The first experiment revealed that neither the acute nor the chronic disease picture described by AHMET is an adequate diagnostic test of invasion by *Fusarium lycopersici* and of its establishment in the tomato plant. Microscopic examination of stems and plate cultures also showed that vascular browning of the stems alone, as found by FISHER, is not an invariant test. Consequently recovery of the fungus from the base of the stems, 3–5 cm. above the sand line, was used as a criterion of infection. All isolations which showed fungous growth were studied microscopically to ensure identification of *F. lycopersici*. In many cases isolations were made from the middle and top of the stem.

Experimental results

SYMPTOMS OF THE DISEASE

It was found that under the experimental conditions imposed, wilting was not the most characteristic symptom of tomato plants invaded by *F. lycopersici*, and that it may be completely absent in plants that die as a result of a slow necrosis of parts.

As pointed out by AHMET, two general disease pictures may be recognized: (a) the "chronic" type (figs. 1–4) in which the disease course is slow, and (b) the "acute" type (fig. 5) in which the disease course is fairly rapid, with true wilting.

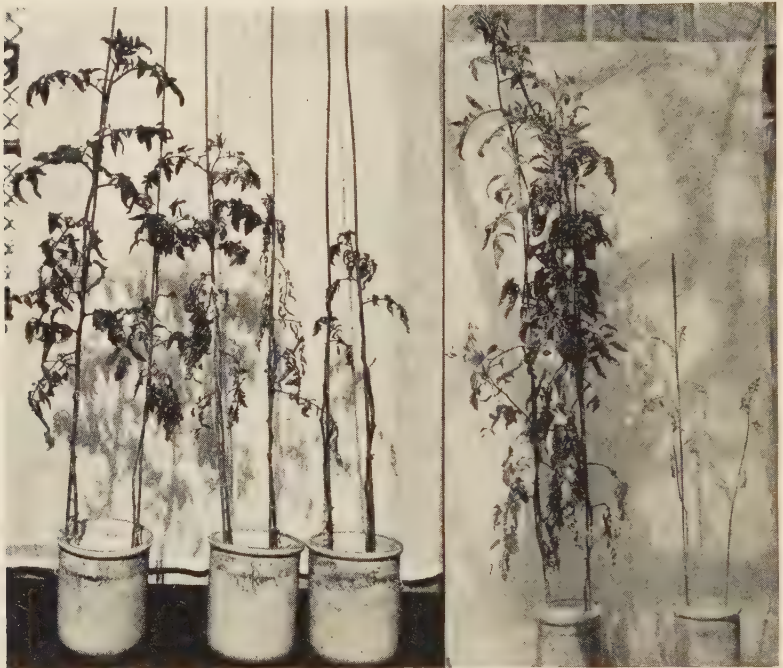
Disease symptoms appearing in the plus nitrate plants were of both the chronic and the acute type, and were abundant in the susceptible plants. Resistant plants as a whole developed few symptoms, these being of the chronic type (fig. 2). True wilt was not observed in the resistant plants.

Infected minus nitrate (high carbohydrate) plants were characterized by the chronic type of disease picture (figs. 3, 4). Symptoms were harder to follow, however, owing to the fact that yellowing and abscission of leaves was constantly taking place in the uninoculated



FIGS. 1-4.—Fig. 1, susceptible Bonnie Best, plus nitrate plants, from experiment IV. Two plants with chronic type of disease shown in culture pot on left. Note absence of lower leaves in righthand plant, also stunted condition of both plants. Control plants in pot at right. Plants under nutritional treatment since April 13, 1936. Photographed May 22. Fig. 2, resistant Marglobe variety under plus nitrate nutrition. Plant at right manifests chronic type of disease and is extremely stunted; lefthand plant apparently healthy but gave, at end of experiment (July 2, 1936), fungus on culturing. Experiment, date of photograph, and cultural conditions same as for fig. 1. Fig. 3, Bonnie Best variety under minus nitrate culture; experiment IV. Plant at left shows chronic type of disease and is stunted; plant at right apparently healthy but gave positive recovery of fungus when cultured at end of experiment. Plants under minus nitrate culture since April 13, 1936. Photographed May 22. Fig. 4, Bonnie Best variety under minus nitrate culture; experiment IV. Plant in righthand pot manifests chronic type of disease symptoms, is stunted, and has lost all leaves except topmost one. Control plants in pot on left. Plants under minus nitrate culture since April 13, 1936. Plants photographed at same time as those shown in fig. 5.

plants. Symptoms of the diseased plants could be recognized, however, for in the uninoculated plants the tops remained green; whereas, in the diseased ones, yellowing involved all of the leaves, until the



FIGS. 5, 6.—Fig. 5 (left), Bonnie Best, plus nitrate plants, from experiment IV. Righthand plant, center pot, shows acute type of disease picture, with definite wilting; lefthand plant, yellowing and necrosis of leaves approximately half way up the stem. Culture pot on right shows two diseased plants which as yet show no wilting. Control plants in culture pot on left. Plants under nutritional treatment since April 13, 1936. Photographed June 3. Fig. 6 (right), representative control plants of experiment I showing effects of plus and minus nitrate nutrition on vegetative growth of tomato plants. Plus nitrate plants in culture pot on left, minus nitrate (high carbohydrate) plants on right. Photographed July 1, 1935.

plant was finally denuded of all foliage (fig. 4). Stunting of the plants was usually another definite symptom which could be used to differentiate between healthy and diseased plants (figs. 3, 4). In a few cases some of the plants showing the chronic type of symptoms recovered. Both resistant and susceptible minus nitrate plants manifested the same chronic type of disease picture.

In addition to the regular chronic and acute symptoms noted, additional ones were observed. Cessation of growth in the apical meristems (stunting), already mentioned, was a striking feature, and in most cases of regular occurrence (figs. 1-4). In fact growth measurements made at weekly intervals often identified diseased plants before other symptoms were evident. Another striking symptom occurring in many cases as a part of the chronic disease picture, and only in the plus nitrate plants, was that of the intense greening of the topmost leaves. Plants in this stage presented a striking appearance. They were stunted, with the intensely dark green leaves flaccid and slightly drooping but not wilted. This incipient flaccidity of the leaves sometimes (but not usually) developed into a true wilt, death of the leaves coming about through slow necrosis.

In connection with some fumigation in the greenhouses, plants in experiment IV were given two applications of cyanide to control the white fly. After both fumigations it was noticed that the upper leaves were severely burned. The leaves of those plants which showed definite *Fusarium* symptoms (plus nitrate plants), however, were entirely free from cyanide injury. No attempt has been made to explain this observation. Possibly the explanation lies in AH-MET's observations that the rate of transpiration in infected tomato plants was much less than in healthy ones, and that in addition, the diseased plants had their stomata closed.

Plants in experiment IV were checked for the presence or absence of adventitious roots, relative to FISHER's observation that such roots occur on the stems of diseased tomato plants. His observations were found to be diagnostic for plus but not for minus nitrate plants. Adventitious roots have also been noted on diseased petioles of plus nitrate plants.

BROWNING OF VASCULAR BUNDLES AS CRITERION OF INFECTION

Various investigators have noted definite browning of the vascular bundles of stems of affected plants and many have used it as a criterion of infection. In the course of the different experiments, observations were made on the presence or absence of vascular browning at the base of the stems. An attempt has been made to

correlate these results with the infection results, which are based on the isolation of the fungus from the stem base.

Results given in table 2 show that out of a total of 275 plants, 144 gave definite browning of the vascular bundles, with 138 (95.8 per cent) positively infected, and 131 gave no evidence of brown vascu-

TABLE 2
BROWNING AND NON-BROWNING OF VASCULAR BUNDLES
AS CRITERION OF INFECTION

MATERIAL	CONDITION OF VASCULAR BUNDLES IN RELATION TO INFECTION								
	TOTAL NO. PLANTS	BROWN BUNDLES AT BASE			NO BROWN BUNDLES AT BASE			TOTAL INFECTED	
		No.	NUM- BER IN- FECT- ED*	PER- CENT- AGE IN- FECT- ED*	No.	NUM- BER IN- FECTED	PER- CENT- AGE IN- FECTED	No.	PER- CENTAGE WITH BROWN BUNDLES
									PER- CENTAGE WITH NO BROWN BUNDLES
Bonnie Best + nitrate.....	69	54	52	96.3	15	5	33.3	57	91.2
Marglobe + ni- trate.....	70	36	33	91.7	34	4	11.8	37	89.0
Total.....	139	90	85		49	9		94	
Average.....				94.4			18.3		90.4
Bonnie Best - nitrate.....	66	31	31	100.0	35	17	48.6	48	64.6
Marglobe - ni- trate.....	70	23	22	95.6	47	12	25.5	34	64.7
Total.....	136	54	53		82	29		82	
Average.....				98.1			35.4		64.6
Grand total.....	275	144	138		131	38		176	
Average.....				95.8			29.0		78.4

* Infected means presence of fungus in tissues of host.

lar bundles. Of these, 38 (29.0 per cent) were infected, however; that is, they yielded fungus upon culturing. One hundred seventy-six infected brown bundle plants and infected non-brown bundle plants were found; 78.4 per cent of these had brown bundles, and 21.6 per cent, no brown bundles. A comparison of the amount of bundle browning and the amount of infection resulting shows no ap-

preciable differences, either for the two varieties or for the different kinds of nutrition. Practically all (95.8 per cent) of the isolations from the base of the stems with brown bundles gave positive infections.

In those plants showing no brown bundles at the base, however, results are different. Minus nitrate plants showed high infection (35.4 per cent), while in the plus nitrate plants only 18.3 per cent of the plants with no browning of vascular bundles were infected.

Nutritional experiments

EXPERIMENT I (TABLE 3)

On March 29, 1935, cuttings of Bonnie Best and Marglobe varieties of tomatoes were transplanted into sterilized sand. The plants were fairly succulent and gave a distinct test for nitrates. Five days after transplanting, all cuttings received plus nitrate solution and were grown continuously under plus nitrate culture for the next 24 days (period ending April 22). At the end of this period approximately half of the plants of each variety were transferred to minus nitrate culture. At the time of changing cultural conditions the average height of all plants was 23.1 cm.

On May 7, all plants were inoculated by pouring fungous inoculum around the roots. At the time of inoculation the plus nitrate plants (especially the tops) were growing vigorously and were fairly succulent. The stems were large and stocky, with an average height of about 53.5 cm. In contrast to the plus nitrate plants, the minus nitrate plants were much less vegetative and succulent, the lower leaves beginning to turn yellow, shrivel, and absciss. The stems were hard, yellowish green in color, and of small diameter. These plants were becoming typical, hard, high carbohydrate plants; they averaged 45.4 cm. in height (fig. 6).

During the elapsed period, from the time of inoculations (May 7) to the end of the experiment (August 1), all plants which exhibited definite *Fusarium* symptoms were removed and used for petri dish isolations. At the end of the experiment (72 days from time of inoculation to removal) all plants were cut off about 5 cm. above the surface of the sand, measured, descriptive notes taken, and they were then brought into the laboratory for isolation studies. In all

TABLE 3

MATERIAL	CONTROLS		INOCULATED PLANTS															
	NUM-BER	AVER-AGE HEIGHT (CM.)	WITH EXTERNAL SYMPTOMS*					WITHOUT EXTERNAL SYMPTOMS†					NOT IN-FECTED§		TOTAL IN-FECTION†			
			TOTAL NO. PLANTS	NUM-BER	PER-CENT-AGE OF TOTAL	AVER-AGE HEIGHT (CM.)	INFECTED‡		NUM-BER	PER-CENT-AGE OF TOTAL	AVER-AGE HEIGHT (CM.)	NUM-BER	PER-CENT-AGE (CM.)					
							NUM-BER	PER-CENT-AGE										
														NUM-BER		PER-CENT-AGE		
																	NUM-BER	PER-CENT-AGE
No. I, post-inoculated, May 7-August 1																		
Bonnie Best + nitrate...	6	182.3	24	4	16.7	132.7	4	100.0	20	83.3	221.0	14	70.0	210.0	6	177.0	18	75.0
Bonnie Best - nitrate...	5	86.0	24	0			0		24	100.0	85.4	15	62.5	84.7	9	86.6	15	62.5
Marglobe + nitrate.....	6	181.3	24	0			0		24	100.0	187.0	1	4.2	157.0	23	188.3	1	4.0
Marglobe - nitrate.....	5	105.0	24	0			0		24	100.0	91.0	11	45.8	88.5	13	93.2	11	45.8
No. II, post-inoculated, August 15-October 25																		
Bonnie Best + nitrate...	4	222.0	12	8	66.7	99.4	8	100.0	4	33.3	221.5	3	75.0	213.3	1	246.0	11	91.7
Bonnie Best - nitrate...	2	50.5	9	0			0		9	100.0	52.8	7	77.8	52.4	2	54.0	7	77.8
Marglobe + nitrate.....	4	206.0	12	0			0		12	100.0	228.7	8	66.7	224.6	4	234.3	8	66.7
Marglobe - nitrate.....	2	40.5	12	0			0		12	100.0	50.7	1	8.3	37.0	11	51.9	1	8.3

* Characteristic external symptoms of Fusarium injury.

† Apparently healthy plants without externally detectable Fusarium injury, but not without vascular browning.

‡ Infected means presence of fungus in tissues of host.

§ Not infected means no fungus recoverable by culture on agar plate.

|| Substrate inoculated after transplanted plants had rooted.

TABLE 3—Continued

MATERIAL	CONTROLS		INOCULATED PLANTS																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																									
	NUM- BER	AVER- AGE HEIGHT (CM.)	TOTAL NO. PLANTS	WITH EXTERNAL SYMPTOMS*					WITHOUT EXTERNAL SYMPTOMS†					NOT IN- FECTED§		TOTAL IN- FECTION‡																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																												
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No. III, pre-inoculated,¶ October 18–January 10

Bonnie Best + nitrate...	3	131.0	9	5	55.6	19.2	5	100.0	4	44.4	138.0	1	25.0	143.0	3	133.7	6	66.7
Bonnie Best – nitrate...	2	59.0	9	4	44.5	15.0	0		5	55.5	36.2	0			5	36.2	0	
Marglobe + nitrate....	3	125.0	10	0			0		10	100.0	119.8	7	70.0	118.4	3	123.0	7	70.0
Marglobe – nitrate....	2	36.0	10	1	10.0	27.0	0		9	90.0	38.1	1	11.1	48.0	8	36.9	1	10.0

No. IV, pre-inoculated,¶ April 13–July 2

Bonnie Best + nitrate...	.6	111.0	24	15	62.5	42.6	14	93.6	9	37.5	93.9	8	88.9	90.1	1	124.0	22	91.7
Bonnie Best – nitrate...	6	30.9	24	15	62.5	4.5	15	100.0	9	37.5	22.9	9	100.0	22.9	0		24	100.0
Marglobe + nitrate....	6	100.8	24	7	29.3	41.4	7	100.0	17	70.7	78.3	14	82.5	75.3	3	92.7	21	87.5
Marglobe – nitrate....	6	24.3	24	10	41.7	2.9	9	90.0	14	58.3	15.7	12	85.7	16.3	2	12.3	21	87.5

¶ Substrate inoculated prior to transplanting of plants.

cases isolations were made from the bottom of the stem; in some instances from the middle and the top as well. All isolations were incubated at 28° C., and studied microscopically.

EXPERIMENT II (TABLE 3)

Starting July 15, 1935, cuttings of Bonnie Best and Marglobe were transplanted into sterilized sand. The cuttings were from plants growing in soil in flats. Owing to the crowded condition in the flats, the plants were spindly (approximately 45 cm. in height), with most of their lower leaves abscised. The upper portions of the plants were vegetative and fairly succulent. For the cuttings, only the upper 25 cm. of the plants was used. About a week after transplanting, the individuals of each variety were divided into approximately two equal lots, one lot of each variety being grown under plus nitrate and the other under minus nitrate culture. The set-up of this experiment varied from the preceding one, in that the experimental plants, which were to be used later for the high carbohydrate, minus nitrate plants, received no nitrates after being transplanted. For the next 30 days (July 15 to August 15) all plants were grown under the cultural conditions already outlined.

Starting August 15, all plants were inoculated as in experiment I (post-inoculated). The plus nitrate plants averaged 48.9 cm. in height, were vegetative, green, and very succulent; the minus nitrate plants averaged 20.1 cm. in height, were spindly, with woody stems and small lower leaves that were turning yellow and beginning to absciss, and with light green and small top leaves. In appearance these plants were typically high carbohydrate (fig. 7).

From the time of inoculation to the end of the experiment (72 days, ending October 25), the plants were carefully watched for symptoms of *Fusarium* damage. At the end of the experiment all plants were removed for isolation studies. In all cases isolations were made from the bottom of the stem, and in this experiment from the middle and top of the plant as well. All fungous growth was checked microscopically.

EXPERIMENT III (TABLE 3)

This experiment and the following differ from the preceding in that cuttings were transplanted directly into sand previously inocu-

lated with *F. lycopersici* (pre-inoculation). This procedure shortened the incubation period.

Sand was inoculated on August 23, 1935, and the fungus allowed to incubate until October 18 (55 days). During this period the inoculated sand was watered once daily with tap water, and in addition, each of the inoculated pots received one quart of plus nitrate nutrient solution at weekly intervals.



FIG. 7.—Growth of tomato grown in plus and minus nitrate culture (experiment II). Plus nitrate plants on right, minus nitrate plants on left. Plants under nutritional treatment since July 15, 1935. Photographed August 19.

On October 18, cuttings of Bonnie Best and Marglobe were transplanted into the inoculated sand. Plants used as cuttings for the minus nitrate cultures averaged about 45 cm. in height, and were fairly hard near the base. In making these cuttings only the upper 25 cm. of the plant was used. Plants used in the plus nitrate cultures averaged 25–30 cm. in height, were younger than those used in the minus nitrate pots, and more succulent and vegetative. About one week after transplanting, the plants of each variety were divided into approximately two equal lots, one of which was grown under plus nitrate and the other under minus nitrate culture. In this experiment, as in experiment II, plants growing under minus nitrate culture received no nitrates after transplanting; after which the

plants grew well. Those receiving nitrates became typical plus nitrate plants while those receiving no nitrates became typical high carbohydrate plants.

Plants were carefully watched for *Fusarium* symptoms from the time of inoculation (October 18) to end of the experiment (84 days, ending January 10, 1936). As in previous experiments, isolations were made from the bottom of the stems in all cases, and in this experiment mostly from the middle also; a few isolations were made from the top of the stem as well.

EXPERIMENT IV (TABLE 3)

As in experiment III, cuttings were transplanted directly into inoculated sand.

The sand was inoculated on April 2, 1936, and the fungus allowed to incubate in it for 12 days. During this period all pots containing the fungus were watered once daily with tap water, and in addition each pot received two quarts of plus nitrate nutrient solution.

On April 13, cuttings of Bonnie Best and Marglobe were transplanted into the inoculated sand. Plants used for cuttings were growing in small pots and averaged 20–25 cm. in height. These plants were highly vegetative and succulent, except near the base. They were blooming, and the bottom leaves were beginning to turn yellow and absciss. They gave a distinct test for nitrates and a rather weak test for starch. Plants were prepared for cuttings in the usual manner. Five days after transplanting, the plants of each variety were divided into two equal lots, one-half of each lot being grown under plus nitrate and the other under minus nitrate culture. Plants grown under minus nitrate culture received no nitrates after transplanting; after which the plants made good growth.

Plants were carefully checked for *Fusarium* symptoms from the time of inoculation to the end of the experiment (79 days, April 13 to July 2). Those plants which showed definite enough symptoms to warrant a tentative diagnosis of the disease were removed during the course of the experiment and used for isolation studies. At the end of the experimental period, all remaining plants were removed and used for isolations from the base of the stem. In addition, isola-

tions were made from the middle and top of the stem as well for most plants.

Results obtained from experiments I to IV show the Bonnie Best (susceptible) plus nitrate plants to be highly infected under both types of inoculation at all seasons of the year. The average infection for all plants in the four experiments amounted to 82.6 per cent; 46.4 per cent displayed symptoms.

Infection in the Marglobe (resistant) variety, under plus nitrate culture, also was high in all except experiment I, in which only 4.0 per cent infection was noted. While an average infection of 52.9 per cent was noted in all experiments, only 4.0 per cent of the plants in experiment I were infected. Only 10.0 per cent of all the inoculated plants showed symptoms, all of which were obtained in experiment IV.

Under minus nitrate culture, the percentage of infection was high for the Bonnie Best variety in all except experiment III, which gave no infection at all. The average infection for all of the inoculated plants was 69.7 per cent. Of the post-inoculated (experiments I and II) plants, 66.7 per cent were infected, while 75.8 per cent of the pre-inoculated (experiments III and IV) were infected. Only 19 plants (28.8 per cent) of all the inoculated plants showed symptoms, 15 of which occurred in experiment IV. In experiment III, four plants out of a total of nine exhibited symptoms, but did not yield the fungus when cultured.

Of inoculated plants of minus nitrate Marglobe variety, 48.6 per cent were infected. In experiment I, with post-inoculated plants, 45.8 per cent were infected. In experiment IV, with pre-inoculated plants, 87.5 per cent were infected. In experiments II and III, an average infection of only 9.1 per cent was obtained. In experiment II the plants were post-inoculated and the period extended from August to October, while in experiment III the plants were pre-inoculated, the period extending from October to January. Production of symptoms was low in these plants; only 11 (15.7 per cent) of the inoculated plants developed symptoms, with all but 1.4 per cent occurring in experiment IV. Two plants, one each from experiments III and IV, exhibited symptoms but were negative when cultured.

An average incubation time of 44 days was noted in the post-in-

oculated plants, while an average incubation time of 25.5 days was noted in the experiments in which the plants were put directly into inoculated sand.

SEEDLING TESTS (TABLE 4)

On October 19, 1935, seeds of Bonnie Best and Marglobe were placed in sand which had previously been inoculated, on August 23. From the time of planting to the end of the experiment, lots of each

TABLE 4

INFECTIVITY OF *FUSARIUM LYCOPERSICI* TO BONNIE BEST (SUSCEPTIBLE) AND MARGLOBE (RESISTANT) TOMATO SEEDLINGS GROWN IN INOCULATED SAND UNDER PLUS NITRATE AND MINUS NITRATE CULTURE

MATERIAL	INOCULATED PLANTS													TOTAL INFECTION	
	TOTAL NO. PLANTS	WITH SYMPTOMS					WITHOUT SYMPTOMS								
		INFECTED*					INFECTED* NOT INFECTED†								
		No.	PER-CENT-AGE			No.	PER-CENT-AGE			No.	PER-CENT-AGE	No.	PER-CENT-AGE		
				No.	PER-CENT-AGE			No.	PER-CENT-AGE						
Bonnie Best + nitrate.....	55	19	34.5	5	26.3	36	65.5	3	8.3	33	91.7	8	14.5		
Bonnie Best - nitrate.....	40	25	62.5	16	64.0	15	37.5	2	13.3	13	86.7	19	47.5		
Marglobe + nitrate.....	63	30	47.6	20	66.7	33	52.4	7	21.2	26	78.8	27	42.8		
Marglobe - nitrate.....	74	21	28.4	13	61.9	53	71.6	1	1.9	52	98.1	14	18.9		

* Infected means presence of fungus in tissues of host.

† Not infected means no fungus recoverable by culture on agar plates.

variety were grown in plus as well as in minus nitrate culture, the latter receiving no nitrates during the experiment.

Seeds of both varieties germinated well under the two types of nutrition. Following germination, the seedlings made good growth, the plus nitrate ones being the larger. On November 5 the seedlings were thinned to a uniform distribution in the culture pots. Seedlings were watched daily for *Fusarium* symptoms, and plants showing such symptoms were removed and cultured.

Healthy plus nitrate seedlings made the greater growth. The plus nitrate seedlings were taller, averaged 4.1 cm. in height, with about three true leaves. They were very succulent, with weak stems, and had a tendency to fall over. On the other hand the minus nitrate plants were stiffer and stood up much better, averaged 2.6 cm. in height, and usually produced only one true leaf. The experiment was terminated during the period from December 7 to 10, and all remaining seedlings were removed and used for isolations.

Of the inoculated Bonnie Best plus nitrate plants, 14.5 per cent were infected, with 34.5 per cent developing symptoms.

Of the inoculated Marglobe plus nitrate plants, 42.8 per cent were infected, with 47.6 per cent of the seedlings showing symptoms.

In the Bonnie Best minus nitrate inoculated plants, total infection amounted to 47.5 per cent, with 62.5 per cent of the inoculated individuals showing symptoms.

The Marglobe minus nitrate inoculated seedlings gave a total infection of only 18.9 per cent, 28.4 per cent of the inoculated individuals producing symptoms.

Discussion

It is apparent from the data obtained in these experiments that the so-called susceptibility and resistance of the tomato to *Fusarium* wilt has at least two aspects. One of these is a positive or a negative disposition to infection, that is, to entry of the parasite into the host and its subsequent establishment and spread; the other, a positive or a negative disposition on the part of the tomato to manifest the structural, processal, and behavioral symptoms characteristic of the disease. The former aspect is a measure of the infectivity or non-infectivity of the parasite; the latter of its virulence or avirulence (7).

It is also evident from the results of these experiments that disposition of the tomato to manifest disease symptoms is not unitary. Some infected plants show both vascular discoloration and other symptoms. Others show neither vascular discoloration nor any other disease symptom. Therefore browning of vascular tissues, frequently used as a criterion, is not a measure of the disposition of the tomato to infection.

Because these two aspects of disposition are not clearly differen-

tiated, and because the term infection is indiscriminately used to designate the disease, or some manifestation of symptoms, as well as the state of being contaminated (7), it is difficult to interpret much of the literature dealing with tomato wilt and other wilt diseases.

For the experiments reported here, infection means the state of being contaminated by *Fusarium lycopersici*. It means that the fungus has entered the host, established itself, and spread from the original site of establishment. It does not mean wilt disease in its usual sense. Possibly infection so interpreted involves some injury to cells, so that considered at the cellular level of biological organization, infection of tomato by *F. lycopersici* probably is characterized by microscopically detectable injury and injury reactions; that is, by disease.

The experimental data show that the expression of each of the two aspects of disposition of the tomato is affected by nutrition in both the so-called susceptible and resistant varieties.

CLAYTON (3) states that susceptible varieties of tomatoes grown under minus nitrate conditions "were not infected." If by this he means the fungus was not recoverable in culture, the results of this investigation are at variance with his, because susceptible Bonnie Best plants grown under minus nitrate nutrition yielded fungus upon culturing, and also developed symptoms in one experiment (IV). These results also are at variance with FISHER's (VI), who used browning as a criterion of susceptibility of infection, and reports that the Bonnie Best variety was characterized by lack of infection when grown in absence of nitrates.

FISHER reports that the resistant Marglobe showed practically no infection when grown in absence of nitrates. The results of this study are at variance with his, in that in two experiments (I and IV) Marglobe showed infection when grown without nitrates. FISHER reports that Marglobe developed infection when grown with an excess of nitrates. In the experiment reported here, frequency of infection was high in both the resistant and susceptible varieties (except in experiment I) when grown in plus nitrate nutrition. In experiment I Marglobe was resistant to infection.

The writer is unable to advance any plausible explanation for the exceptions noted in experiments I-IV. Average frequency of infec-

tion of Marglobe plants was higher when the plants were transplanted into pre-inoculated sand (experiments III and IV) than when inoculation was effected by working the fungus into the sand around established plants (experiments I and II). Possibly severe trauma and other injuries incident to cutting and transplanting disposed the plants to infection. Bonnie Best also showed a higher average frequency of infection in the experiments (III and IV) involving cutting and transplanting into previously inoculated sand. It is possible that the amount of fungus present was greater and more effective in the pre-inoculated sand experiments. Possibly the age of the plants also contributed to injury. These transplanted plants were younger and more succulent at the base than the established plants. Possibly differences in air temperature, humidity, and sunshine were factors, through different influences on the nutritional state of the experimental plants at different seasons of the year. Generally speaking, both plus and minus nitrate plants contain more carbohydrates (starch) during the late spring, summer, and early fall months than during the winter months. Plus nitrate plants are high in nitrates at all seasons of the year, while minus nitrate plants contain no readily demonstrable nitrates at any season of the year.

I am also not prepared to explain the differences in frequency of expression of symptoms noted in experiments I-IV. Only Bonnie Best plants when post-inoculated were disposed to symptom manifestation in plus nitrate culture. In pre-inoculation experiments, however, both Bonnie Best (in experiments III and IV) and Marglobe (in experiment IV) were disposed to symptom manifestation in plus nitrate culture. The former alone manifested true wilting in addition to the chronic type of symptoms; the latter manifested only the chronic type. The same factors that were responsible for irregularities in the infection data may have been responsible for deviations in the manifestation of symptoms. Pre-inoculations and post-inoculations also affected the incubation time, symptoms appearing in 25 days for the first and in 44 days for the latter.

These experiments reveal discrepancies between frequency of browning in the stem or manifestation of other symptoms, and between frequency of recovery of the fungus by culture. Plants with brown bundles (experiments III and IV) did not invariably yield the

fungus in culture. This does not mean that they were not infected, because presence or absence of the fungus in the root system was not determined by culture. It is well known that browning may occur in advance of the site of the fungus. A decisive answer to this problem would entail culture of the entire root system of every plant.

The other discrepancy, namely the recovery of fungus from plants showing no symptoms whatsoever in the aerial parts, indicates manifestation of low virulence on the part of the parasite and of high resistance to virulence on the part of the plant. This discrepancy was most marked in plants under minus nitrate nutrition.

Possibly the discrepancy between infection as determined by culture on the one hand and by browning on the other would have been less if microscopically detectable browning had been used as a criterion of presence of the fungus. FISHER used the microscopic criterion.

Summary

1. Experiments were conducted to study the relation of nutrition of the tomato as a factor in its disposition to the infectivity and virulence of *Fusarium lycopersici*. Bonnie Best (susceptible) and Marglobe (resistant) varieties were tested. Nutrition was varied by the application or non-application of nitrates under otherwise identical conditions. The substrate was inoculated both previous (pre-inoculation) to and subsequent (post-inoculation) to the establishment of the plants.

2. Recovery of the fungus from the base of the stem was used as a criterion of infectivity and infection. Manifestation of macroscopically detectable symptoms was used as a criterion of virulence and of pathic effects in the host.

3. Results of the nutritional experiments revealed (a) a high frequency of infection of both resistant and susceptible tomato plants under minus nitrate nutrition; (b) a low frequency of symptoms in plants under minus nitrate nutrition; (c) no wilting in the so-called resistant Marglobe under either type of nutrition; (d) seedlings of the resistant variety under either plus or minus nitrate culture read-

ily infected and producing symptoms identical with those of the susceptible variety.

4. Type of inoculation and age of the plant are important factors in infection and symptom production: (a) Pre-inoculation of sand led to a higher frequency of infection and symptom production. (b) Post-inoculation of sand under minus nitrate culture did not lead to the development of any plant symptoms, even in infected plants. (c) Bonnie Best variety under plus nitrate nutrition showed a high frequency of infection and of symptom production of both the chronic and the acute type, at all seasons of the year, when either pre-inoculation or post-inoculation was used. (d) Pre-inoculation practically halved the incubation period.

5. Browning of vascular bundles of the stem alone is an inadequate criterion of infection. Even under minus nitrate nutrition, 35.4 per cent of the infected plants did not manifest brown bundles at the base of the stem.

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LITERATURE CITED

1. AHMET, K., Untersuchungen über Tracheomykosen. *Phytopath. Zeitschr.* 6:49-101. 1933.
2. CLAYTON, E. E., The relation of temperature to the *Fusarium* wilt of the tomato. *Amer. Jour. Bot.* 10:71-88. 1923.
3. ———, The relation of soil moisture to the *Fusarium* wilt of the tomato. *Amer. Jour. Bot.* 10:133-147. 1923.
4. EDGERTON, C. W., A study of wilt resistance in the seed-bed. *Phytopath.* 8:5-14. 1918.
5. EDGERTON, C. W., and MORELAND, C. C., Tomato wilt. *Louisiana Agr. Exp. Sta. Bull.* 174. 1920.
6. FISHER, P. L., Physiological studies on the pathogenicity of *Fusarium lycopersici* Sacc. for the tomato plant. *Maryland Agr. Exp. Sta. Bull.* 374. 1935.
7. LINK, G. K. K., Etiological phytopathology. *Phytopath.* 23:843-862. 1933.
8. ———, The Chicago soil-nutrient-temperature tank. *Science n.s.* 81:204-207. 1935.

